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ASSISTED REPRODUCTION TECHNOLOGIES

Switching to testicular sperm after a previous ICSI failure with ejaculated sperm significantly improves blastocyst quality without increasing aneuploidy risk

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Abstract

Purpose The use of testicular sperm is confined to patients with azoospermia, but there is evidence to support its use in males with poor semen parameters and/or previous intracytoplasmic sperm injection (ICSI) failures with ejaculated spermatozoa. We compared the aneuploidy rate and quality between embryos derived from ICSI cycles with ejaculated sperm (EJ-ICSI) and those from ICSI cycles using testicular spermatozoa (TT-ICSI) within the same couple.

Methods Retrospective study of 27 couples who first underwent an EJ-ICSI cycle that did not result in a livebirth and afterwards a TT-ICSI cycle. Only the two closer cycles of each couple were included. Preimplantation genetic test for aneuploidies (PGT-A) was performed in both ICSI cycles and classic parameters of embryo quality were assessed until blastocyst-stage.

Results A total of 375 embryos from 54 ICSI cycles were evaluated. Aneuploidy rate was measured by two different parameters. Patients undergoing TT-ICSI presented a similar aneuploidy rate as EJ-ICSI group: 30.7% (23.4–38.0) vs 26.8% (18.1–35.5) per inseminated oocytes ($P>0.05$), and 76.2% (66.2–86.2) vs 72.1% (59.1–85.2) per the total number of biopsied embryos ($P>0.05$), respectively. Further, the good-quality blastocyst rate per correctly fertilized oocyte was significantly higher in TT-ICSI group (33.6% (30.4–36.9)) than EJ-ICSI group (24.2% (20.3–28.0)) ($P<0.001$).

Conclusions Switching to testicular sperm for ICSI yielded better-quality blastocysts without affecting the chromosomal load of the embryos in non-azoospermic couples with a previous unsuccessful ICSI using ejaculated sperm. This strategy is a good option for couples seeking a livebirth who do not want to use donor sperm.

Keywords Aneuploidy rate · Ejaculated sperm · ICSI · Male factor · Testicular sperm

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Introduction

Since its first description in 1992, intracytoplasmic sperm injection (ICSI) has been widely used to overcome all forms of severe male infertility [1], requiring only a low number of sperm to fertilize the oocytes. In addition, the combination of surgical extraction of testicular spermatozoa with ICSI technique was a revolution for those men with azoospermia to achieve biological paternity [2–4], obtaining relatively acceptable success rates and demonstrating the innocuousness of the technique for the male and the offspring obtained [5].

The use of testicular sperm is mandatory when sperm are not found in the ejaculate. However, its use has also been proposed recently as a clinical strategy in cases of males with sufficient sperm in the ejaculate but showing severe male infertility [6–9] or high DNA fragmentation values

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[10–12], and even in couples experiencing unsuccessful ICSI cycles (understood as failure to achieve a live birth) to increase the chances of pregnancy [9, 13].

With the improvement of in vitro fertilization techniques as well as in testicular sperm retrieval methods, this practice has become more common in recent years. Some authors did not find poor ICSI outcomes when using non-ejaculated spermatozoa, not only when comparing the results between ICSI cycles using testicular versus ejaculated sperm [14], but also when evaluating ICSI outcomes using testicular versus epididymal spermatozoa [15]. Significantly superior results were even found when comparing ICSI treatments using testicular sperm with ICSI cycles using ejaculated spermatozoa in terms of implantation rate (36.8% versus 19.9%) and pregnancy rate (23.7% versus 12.7%) in patients with necrozoospermia [16], and in those couples with male factor and long-term infertility [9]. Indeed, in this little case-report study concluded that changing the sperm source for ICSI in four couples with recurrent implantation failure, several previous unsuccessful IVF/ICSI cycles, and no other obvious cause of male infertility improved their clinical outcomes obtaining all couples a newborn.

Likewise, the use of testicular-retrieved sperm in males with increased DNA fragmentation in their ejaculated sperm seems to enhance the probability of a live newborn. This reasoning is based on the fact that testicular spermatozoa have less genome damage due to its low exposure to reactive oxygen species unlike sperm storing and transporting along the epididymis and genital tract [17–19]. There are both prospective and retrospective studies that demonstrate improved outcomes when testicular sperm were used [10, 11, 18–20]. One meta-analysis [21] comprising data from 507 ICSI cycles of patients with oligozoospermia and high sperm DNA fragmentation found that the live newborn rate was 46.9% in couples using testicular sperm, 21% higher than the live newborn rate obtained in patients who underwent ICSI with ejaculated sperm cells (21.3%).

By contrast, other authors stated that ejaculated spermatozoa lead to better clinical results due to their maturity, since during their passage through the epididymis they acquire modifications necessary for their subsequent encounter with the oocyte and its fertilization [22]. Since testicular sperm does not undergo these changes in the epididymis, this would explain why fertilization, implantation, and pregnancy rates were lower along with a higher probability of miscarriage in ICSI cycles using testicular sperm instead of epididymal sperm [23, 24] or compared to those using ejaculated sperm [5, 7]. As a matter of fact, the latest meta-analysis in 2018 [25] questions the benefits of switching to testicular sperm. They evaluated whether the pregnancy likelihood is higher in ICSI cycles performed with testicular spermatozoa in males with abnormal semen parameters. The main conclusion reached was that the evidence is of low quality and limited to recommend the use of testicular spermatozoa instead of ejaculated ones to improve the pregnancy outcomes in males with oligozoospermia and high levels of sperm DNA fragmentation.

Furthermore, an increased risk of sperm chromosome aneuploidies has been related to males with severe infertility [26–30] and with obstructive and non-obstructive azoospermia [31, 32], as a possible result of impaired spermatogenesis. In addition, the rate of aneuploidy was found higher in testicular spermatozoa than in ejaculate ones when compared from the same individual [33]. The total aneuploidy rate was significantly higher the lower the sperm concentration, being an inverse correlation and more evident in males with severe oligozoospermia [28, 29]. Based on this premise, it should be considered that chromosomal abnormalities of the spermatozoon may affect the genetic load of the future embryo [27, 28] and, in consequence, its quality [34, 35] in couples with severe male infertility.

Euploid and good-quality embryos are unequivocally related with improvement of clinical outcomes. Therefore, the use of testicular sperm should be followed by the evaluation of the chromosomal status of the embryos [36]. The application of preimplantation genetic analysis for aneuploidy (PGT-A) allows the selection of euploid embryos for transfer which lends a higher reproductive success, reducing the incidence of pregnancies and offspring with chromosome abnormalities. However, in terms of embryo quality, there is still no sufficient evidence about how can be affected by testicular retrieved sperm [37–39], and its impact should be further evaluated.

To date, the basis for the better pregnancy outcomes reported from couples that decided to change the sperm origin for their subsequent ICSI attempt is unclear. Although testicular sperm could present better physiological features compared with the ejaculate ones in certain non-azoospermic males, little is known if this improvement derived from genetic or cytoplasmic factors in the embryos obtained. This gap of knowledge should be evaluated before recommending this practice to couples with poor reproductive outcomes with ejaculated semen. The main objective of this study was to compare the aneuploidy rate between embryos derived from ejaculated sperm and embryos derived from testicular sperm from ICSI cycles with PGT-A within the same couples. As a secondary objective, embryo quality was also compared between the two groups.

Materials and methods

Study population

This study included the clinical data from 27 couples who had at least one previous unsuccessful ICSI cycle,

understood as the failure to obtain a live birth after transferring all available embryos, using ejaculated sperm (EJ-ICSI) that subsequently underwent an ICSI with spermatozoa obtained from testicles (TT-ICSI) between January 2000 and November 2020. Only the two ICSI cycles closest in time were included with the aim of standardizing and controlling the characteristics of the patients in both treatments. A PGT-A was performed in both ICSI cycles using autologous oocytes. Only ICSI cycles performed with ejaculated semen prior to ICSI-TT were included.

Eligible subjects: i) males with sperm count on their ejaculate; ii) at least one previous ICSI failure using ejaculated sperm; and iii) use of testicular spermatozoa obtained by TESE for ICSI. Males with azoospermia that are unable to ejaculate sperm, couples that changed the origin of the oocytes between the two cycles, and IVF-ICSI (mixed) cycles were excluded. ICSI cycles with donor eggs were not included.

This study was approved by The Ethics Committee for Investigation of the University and Polytechnic La Fe Hospital (project code 2011-FIVI-096-NG).

Sperm collection

Semen samples from ejaculate were collected after 3–5 days of sexual abstinence in a sterile recipient by masturbation. After liquefaction, basic sperm parameters (volume, concentration, motility, and morphology) were evaluated according to the World Health Organization criteria [40].

Surgical sperm retrieval by TESE (testicular sperm extraction) technique was used to collect spermatozoa from testicles in all cases, following the procedure as previously reported [41]. Briefly, after the administration of local anesthesia, the scrotal skin and tunica vaginalis were opened. One small incision was made through tunica albuginea to extrude testicular tissue, which was excised and placed in sperm preparation medium (Medicult; Jyllinge, Denmark). The tissue was dissected manually with sterile slides and the presence of sperm cells under an inverted microscope at ×400 magnification was checked. When an adequate number of spermatozoa was found for ICSI technique, the procedure was ended. Otherwise, another sample was taken from a different region but in the same testicle. Sperm cells were immediately frozen for the later use as described [42, 43].

Ovarian stimulation protocols

Ovarian stimulation was carried out with different protocols, with the majority being Gonadotrophin-releasing hormone (GnRh) -agonist and –antagonist protocols, previously described [44, 45]. They were begun on day 2 or 3 of the menstrual cycle according to the patient's ovarian reserve and requirements and administered until the leading follicles reached a mean diameter of ≥18 mm. Transvaginal ultrasound-guided oocyte retrieval was performed to collect follicles 36 h later. The follicles aspirated were mechanically denuded at 2 h if the oocytes were to be vitrified for later insemination, or at 4 h if they were to be subsequently microinjected [46, 47] after the protocol described elsewhere.

ICSI and laboratory procedures

All couples underwent ICSI, firstly with sperm from ejaculate and then with testicular sperm obtained by TESE. Insemination was performed with either fresh or vitrified-warmed [48] oocytes. ICSI procedure was reported elsewhere [49]. Ejaculated sperm samples were prepared by swim-up or by density gradient as previously reported [50, 51] before ICSI. Sperm injections of EJ-ICSI were performed with either fresh or frozen specimens whereas in TT-ICSI were always with frozen-warmed samples.

The fertilized oocytes were cultured under laboratory conditions (37°C; 6% CO_2; 5% O_2), and zygotes and embryos were evaluated throughout embryonic development. All embryos were subject to a PGT-A at day 3 (removing one or two blastomeres) [52] or at day 5 (removing few cells from trophectoderm) [53] of embryonic development. Embryos biopsied at day 3 were left until day 5 of development to be transferred (fresh ET) or vitrified and subsequently transferred (frozen-thawed, FET) [54], if was applicable. All embryos biopsied at day 5 were vitrified pending the results of the analysis and afterwards transferred (FET). PGT-A analyses were always performed by an external company contracted for this purpose, following standardized protocols for each of the techniques used according to technological advances. The analysis technique to detect aneuploidy was the same in both ICSI cycles.

Euploid embryos transfer took place at day 5 or 6 according to blastocyst stage in all cases. Some patients required luteal phase support prior to embryo transfer or endometrial preparation (hormone therapy (HT) cycles) [55, 56]. The number of embryos replaced complied with Spanish law and patient's requirements and clinical history.

Outcome measures

The main outcome was aneuploidy rate presented as the proportion of the number of embryos categorized after PGT-A as aneuploidies divided by the total number of inseminated oocytes. In addition, it was calculated per the total number of zygotes and per the total number of biopsied embryos. Blastocyst rate was calculated as the number of embryos that developed until full blastocyst stage at day 5 divided by the total number of inseminated oocytes and by the total number of correctly fertilized oocytes. Embryo quality was assessed at day 5 (D5) according to the morphological

criteria established by ASEBIR (Association for the Study of Reproductive Biology) [57]. Blastocyst was assessed following these morphological parameters: i) the degree of blastocyst expansion (cavitation, full expansion, or hatching); ii) trophectoderm (TE) quality (defined as A, B, C, or D) and inner cell mass (ICM) quality (defined as A, B, C or D). The good quality blastocyst rate was the proportion of A and B blastocysts per the total number of inseminated oocytes, per the number of correctly fertilized oocytes, and per the total number of blastocyst stage embryos.

The reproductive outcomes evaluated were implantation rate; biochemical pregnancy rate as the beta-hCG measuring in blood serum higher than 10 IU/L at 10 days after ET; and clinical pregnancy rate described as the detection of heartbeat at 7 weeks' gestation. Ongoing pregnancy rate as the achievement of pregnancy greater than 12 weeks; miscarriage rate as the loss of gestation after positive beta-hCG; and live birth rate, as the birth of a newborn.

Statistical analysis

All data were extracted from electronic medical record. Data analysis was performed using R Software (4.02 version. R Core Team (2020). R Foundation for Statistical Computing, Vienna, Austria). The continuous variables were reported as mean and 95% confidence interval (95% CI). Categorical variables were described as the proportion of cases (%) and 95% CI. Differences between two groups were compared by two-tailed paired *t*-test for data normally distributed and Pearson's Chi-squared test for categorical data. A *P*-value<0.05 was considered statistically significant.

Results

Our study cohort is composed of 27 couples who underwent an EJ-ICSI and a TT-ICSI cycle with autologous oocytes. The interval between the two treatments was 509 days (17 months) (41–1471 days). Mean female and male ages were 36.2 (34.4–37.9) and 40.2 (37.9–42.4) years old in EJ-ICSI group, while it was 37.0 (35.5–38.5) and 41.2 (39.1–43.3) years old in TT-ICSI group, respectively. There were no significant differences regarding demographic and clinical data between the two groups (Table 1).

Female etiology was advanced maternal age (21.4%), anexectomy (14.3%), ovarian polycystic syndrome (14.3%), low responders (7.1%), and uterine factors (7.1%). The others (35.7%) were normal or idiopathic. The semen characteristics of the EJ-ICSI cycles are found in Table 2. Male infertility diagnosis was severe oligozoospermia (4.8%), oligoasthenoteratozoospermia (OAT) (23.8%), asthenozoospermia (23.8%), criptozoospermia (23.8%), and oligoasthenozoospermia (23.8%).

Table 3 presents the laboratory outcomes of both ICSI cycles. The total number of embryos evaluated was 375; of these, 214 embryos were biopsied. There were no significant differences in the fertilization rate or in embryo quality parameters evaluated on day 5 of embryonic development between EJ-ICSI group and TT-ICSI group. As TE mainly marks the final quality of blastocysts, the proportion of TE and ICM quality grade is shown in the Figure 1, but these differences were not statistically significant between the two groups.

Regarding aneuploidy rate, embryos derived from ejaculated sperm ICSI cycles presented a lowered, but not statistically significant, aneuploidy rate compared to embryos derived from retrieved testicular spermatozoa (Table 3) in all approaches performed. Forty euploid embryos were transferred; all couples underwent a single ET at blastocyst stage (at day 5 or day 6). When comparing embryo quality, the proportion of good-quality blastocyst rate was 9% higher in TT-ICSI group when calculated per the total number of correctly fertilized eggs, 33.6% (30.4–36.9) vs 24.2% (20.3–28.0) in EJ-ICSI group (P <0.001). Finally, Table 4 describes the clinical outcomes of TT-ICSI cycles.

Discussion

The use of testicular sperm for ICSI is a clinical strategy that has been employed in the past years to improve reproductive outcomes in couples who have had one or more previous ICSI failures using ejaculated semen, especially in couples in which the male has poor semen quality. Among the reports addressing this approach, an improvement in clinical outcomes has been observed in relation to pregnancy rate, a decrease in miscarriage rate and, therefore, a greater live birth rate. However, if this improvement is achieved through embryo ploidy or non-genetics features remains to be well elucidated to date. Additionally, these studies are few, conducted on heterogeneous populations and many of them do not compare this clinical strategy in the couple itself but in different groups of patients.

To our knowledge, this is the first study in the literature comparing the ploidy and quality of the embryos obtained in ICSI cycles using testicular sperm with ICSI cycles using ejaculated sperm from the same couple. The retrospective evaluation of 54 ICSI cycles from 27 couples revealed a similar proportion of aneuploid embryos in the ejaculated sperm and in the testicular sperm group. Further, we assessed embryo quality, finding a significantly higher good-quality blastocyst rate in those ICSI cycles performed with testicular-retrieved sperm. Of the 27 couples who underwent this procedure, 10 women achieved clinical pregnancy, of which eight resulted in the delivery of at least one healthy newborn. These results are helpful for clinicians to be able

Table 1 Demographics and clinical data of patients per ICSI cycle depending on sperm origin

Parameters	*EJ-ICSI (n=27)*	*TT-ICSI (n=27)*	*P-value*
Female age (y)	36.2 (34.4–37.9)	37.0 (35.5–38.5)	0.3
Female BMI (kg/m^2)	24.0 (22.6–25.4)	23.8 (22.5–25.2)	0.9
Male age (y)	40.2 (37.9–42.4)	41.2 (39.1–43.3)	0.4
Male BMI (kg/m^2)	27.0 (25.1–29.0)	27 (25.1–29.0)	0.7
Antral Follicular Count (AFC)	13.2 (10.5–16.0)	11.7 (5.7–17.8)	0.6
AMH levels	2.5 (5.4–8.8)	2.5 (1.0–4.1)	1.0
Nº stimulation days	11.8 (9.6–14.1)	11 (10.4–11.7)	0.3
FSH total dose (IU)	1951.1 (1673.2–2229.0)	1821.3 (1560.8–2081.8)	0.3
LH total dose (IU)	-	925 (203.2–1646.8)	0.9
HMG total dose (IU)	1051.3 (812.5–1290.0)	998.2 (715.7–1280.7)	0.5
E2 levels on hCG (pg/mL)	2195.4 (1736.4–2654.3)	2150.7 (1732.7–2568.6)	0.8
P4 levels on hCG (pg/mL)	1.0 (0.8–1.1)	1.0 (0.8–1.2)	1.0
Nº days of endometrial preparation	17.6 (14.7–20.6)	17.0 (16.0–18.0)	0.5
Latest endometrial thickness (mm)	9.8 (9.2–10.5)	9.3 (8.4–10.2)	0.2
Latest E2 level (pg/mL)	2174.5 (1641.1–2707.9)	2107.9 (1423.2–1550.1)	0.8
Latest P4 level (pg/mL)	1.0 (0.8–1.2)	1.0 (0.7–1.2)	0.5
Nº aspirated oocytes	12.3 (10.0–14.5)	12.6 (9.8–15.3)	0.9
Nº MII oocytes	11.6 (9.3–14.0)	11.0 (8.4–13.6)	0.7
Nº MI oocytes	0.7 (0.3–1.0)	0.7 (0.4–1.1)	1.0
Oocyte state (%)	-	-	0.5
fresh	77.8 (57.7–91.4)	66.7 (46.0–83.5)	-
vitrified	7.4 (0.9–24.3)	28.5 (6.3–38.1)	-
mixed	14.8 (4.2–33.7)	14.8 (4.2–33.7)	-
Nº of inseminated oocytes	10.9 (8.8–13.1)	10.9 (8.3–13.4)	0.9
Nº of correctly fertilized oocytes	7.1 (5.4–8.8)	6.9 (5.1–8.6)	0.9
Nº embryos obtained	6.6 (5.0–8.3)	7.4 (5.9–8.9)	0.6
Nº biopsied embryos	3.7 (2.8–4.6)	4.0 (3.2–4.8)	0.3
Nº analyzed embryos	3.7 (2.8–4.6)	4.0 (3.2–4.8)	0.3
Nº informative embryos	3.6 (2.8–4.5)	4.0 (3.2–4.8)	0.2
Nº aneuploidy embryos	2.7 (2.0–3.5)	3.0 (2.4–3.7)	0.2

Values are mean or proportions (95% CI). *P*-value were calculated with *t*-student test for means and Chi-square test for proportions

BMI body mass index, *AMH* antimullerian hormone, *FSH* follicle-stimulating hormone, *LH* luteal hormone, *E2* estradiol, *P4* progesterone, *hCG* human chorionic gonadotropin

to advise those patients who obtain poor clinical results with their ejaculated sperm on the election of the subsequent most appropriate reproductive strategy, in order to finally obtain a newborn.

In this study, the male population suffered from poor semen parameters (according to WHO, [58]), presenting some patients severe male infertility (cryptozoospermia or OAT). After oocytes insemination with spermatozoa retrieved from the testis, we found that the number of embryos that became blastocysts in similar to ICSI cycle with ejaculated semen. Nevertheless, when blastocyst morphology was evaluated, a significant improvement (of about 10%) in the proportion of good-quality embryos in the testicular-ICSI cycles when measured from fertilization. This phenomenon may be associated to a better quality of male gametes retrieved from the testicle, which show less damage than those from the ejaculate in this category of patients who presented severe male infertility.

The rationale of using testicular sperm instead of ejaculated ones in non-azoospermic males is the better quality of the former due to a higher integrity of the spermatic chromatin. It was previously demonstrated in an animal model [17] that the passage of sperm through the epididymis and genital tract increases DNA fragmentation due to the presence of reactive oxygen species. Subsequent studies in humans demonstrated that ejaculated spermatozoa present higher levels of DNA fragmentation than those retrieved from testicle [22, 59]. According to available reports [6, 9], post-testicular damage could lead to a decrease in the fertilization rate and in the quality of the embryos generated.

Table 2 Semen characteristics of neat ejaculated samples and after seminal preparation of EJ-ICSI cycles (*n*=27)

Seminal parameters	Mean	95% CI
Before seminal preparation		
Volume (ml)	2.5	1.8-3.2
Sperm concentration (mill/mL)	10.0	2.0-18.0
Sperm motility progressive (%)	4.4	0.1-8.6
Sperm motility non-progressive (%)	11.4	-3.3-26
Sperm immobile (%)	87.1	70.6-99.6
Total motile sperm count (mill)	6.6	-1.1-14.4
After seminal preparation		
Volume (ml)	0.4	0.1-0.6
sperm concentration (mill/mL)	0.9	0.4-1.5
Sperm motility progressive (%)	12.3	1.2-23.3
Sperm motility non-progressive (%)	15.3	-1.5-32.1
Sperm immobile (%)	73.8	54.5-93.0
Total motile sperm count (mill)	0.1	0.0-0.3

Surprisingly, the articles published to date hardly compare the quality between ejaculated- or testicular-sperm–derived embryos [6, 10, 12, 20], knowing that the achievement of embryos of the highest quality enhances the likelihood of having a take-home baby. Only Gilman's study [60] demonstrates improved embryo quality in ICSI cycles using testicular sperm, a finding that we support.

In this sense, several studies described the improvement of ICSI outcomes for patients with high sperm DNA fragmentation (SDF) when testicular spermatozoa are used [10–13, 18, 20, 33, 61]. Overall, significant higher fertilization and pregnancy rates per embryo transfer [10, 11, 13, 20] with lower miscarriages rates [11, 18] were reported with this approach. In one retrospective study [62], half of the couples with SDF who had a previous assisted reproduction treatment (ART) failure obtained a pregnancy with a healthy newborn by changing the origin of the sperm. Esteves' study [12] reported a benefit in ICSI outcomes of switching to testicular sperm in those males with severe oligozoospermia and a SDF than 30%. These results were confirmed in his meta-analysis (507 ICSI cycles for TT-ICSI and EJ-ICSI) [63], where the clinical outcomes were improved in a statistically significant fashion in couples performing TT-ICSI when male partners have high SDF in their ejaculate compared with EJ-ICSI couples. However, one of the latest meta-analysis [25] did not find solid conclusions to endorse this practice in this type of patients. In this study, the SDF level was not retrospectively evaluated due to the lack of this information in the enrolled patients, so we cannot relate it to this phenomenon directly.

Nonetheless, several papers also evaluated the change to testicular sperm for ICSI to overcome male infertility related to poor sperm quality in the ejaculate [6, 8, 9, 64], showing better reproductive outcomes with this

Table 3 Laboratory outcomes of ICSI cycles using ejaculated (EJ-ICSI) or testicular (TT-ICSI) spermatozoa within the same couples

	EJ-ICSI (n=27)	*TT-ICSI (n=27)*	*P-value*
Fertilization rate (%)	65.8 (56.4–75.3)	66.7 (59.2–74.2)	0.7 †
Blastocyst rate (%)			
Per inseminated oocytes	50.7 (40.4–61.0)	50.6 (43.8–57.4)	1.0 *
Per correctly fertilized oocytes	66.5 (56.5–76.5)	66.4% (56.9–71.9)	0.7*
Good-quality blastocyst rate (%)			
Per inseminated oocytes	18.6 (7.3–29.9)	19.8 (11.1–28.5)	0.9 *
Per correctly fertilized oocytes	24.2 (20.3–28.0)	33.6 (30.4–36.9)	**<0.001 ***
Per total number of blastocysts	35.0 (20.4–49.6)	45.8 (34.0–57.5)	0.2 *
Aneuploidy rate (%)			
Per inseminated oocytes	26.8 (18.1–35.5)	30.7 (23.4–38.0)	0.3 †
Per correctly fertilized oocytes	41.7 (28.2–55.2)	46.6 (37.0–56.2)	0.4 †
Per total number of biopsied embryos	72.1 (59.1–85.2)	(66.2–86.2)	0.6 †
Total number of embryos transferred	22/177 (7.6–17.3)	18/197 (5.1–13.2)	0.4 *
Day of embryo transfer (%)			
D5 fresh ET	(20.7–63.7)	33.3 (13.3–59.0)	-
D5 FET	40.9 (20.7–63.7)	55.6 (30.8–78.5)	-
D6 FET	18.2 (5.2–40.3)	11.1 (1.4–34.7)	-

Values are expressed as proportions (percentage) and 95% CI. D5: day 5; D6: day 6; *ET* embryo transfer; *FET* frozen embryo transfer. The variable *total number of embryos transferred* is the proportion of embryos that were transferred by the total number of embryos attained (per ICSI group)

*P-value was calculated by Student *t*-test

†P-value was calculated by Paired Student *t*-test

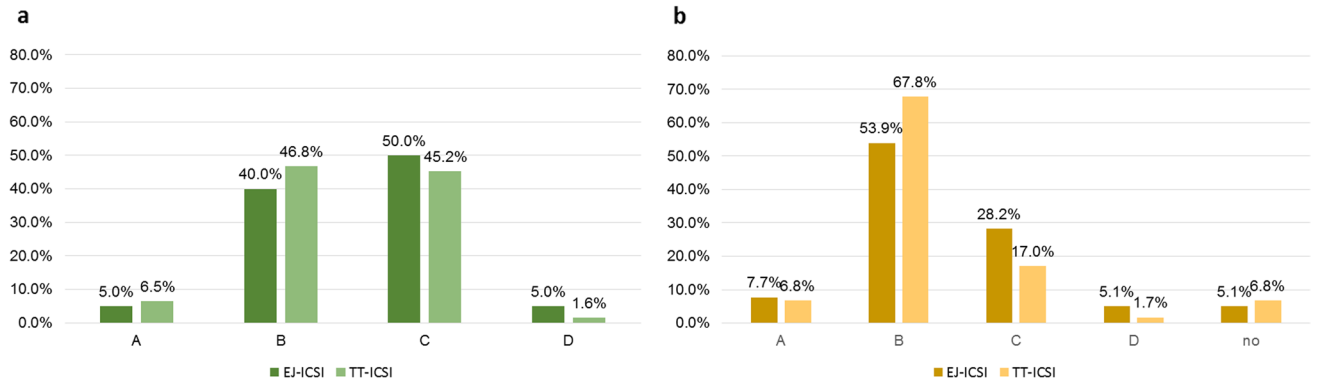

Fig. 1 Embryo quality parameters at day 5 of embryo development corresponding to blastocyst stage. Trophectoderm (TE) (a) and inner cell mass (ICM) (b) characteristics were evaluated and compared between EJ-ICSI and TT-ICSI groups. Chi-squared test: (A) *p*-value= 0.7; (B) *p*-value= 0.3

Table 4 Clinical outcomes after ICSI using testicular sperm (TT-ICSI)

Outcome	Value (%)	95% CI
Implantation rate (*n*)	94.1 (16/17)	15.8–83.7
Biochemical pregnancy rate (*n*)	68.8 (11/16)	41.3–89.0
Clinical pregnancy rate (*n*)	62.5 (10/16)	35.4–84.8
Ongoing pregnancy rate (*n*)	50.0 (8/16)	24.7–75.4
Miscarriage rate (*n*)	27.3 (3/11)	6.0–61.0
Live birth rate (*n*)	50.0 (8/16)	24.7–75.4

Notes: Values are expressed as proportions (percentage) and 95% CI. The clinical outcomes are calculated per cycle

intervention. For instance, cryptozoospermic males benefited from the switch to testicular-retrieved sperm after failure of the previous ICSI cycle with ejaculated sperm. Although no difference in fertilization rate, a significantly higher implantation and pregnancy rate was found in ICSI cycles with testicular sperm of the same patients [6]. They also assessed the quality of the embryos (only at day 2 and day 3 of embryonic development) but no statistically significant differences were seen between two groups. By contrast, this finding was not supported by Abhyankar's meta-analysis of 5 cohort studies [8], in which no differences were found in pregnancy rates after ICSI between the testicular and ejaculated sperm groups of crytozoospermic males. In addition, the testicular-retrieved spermatozoa did not improve the pregnancy, miscarriage, and live birth rates of patients with a previous ART failure [60], although in these cases, the women showed poor ovarian response. Nonetheless, there were significantly more day 5 blastocyst transfers in the testicular sperm group compared to controls, which suggest a better embryo quality. Last but not least, in one case-report [9], the election of testicular spermatozoa for ICSI in males with sperm in the ejaculate suffering long-term infertility and multiple failed IVF/ICSI cycles involved the achievement of ongoing pregnancy/deliveries in the four couples evaluated.

This is an important finding for those couples who are offered to switch to this clinical strategy after several unsuccessful EJ-ICSI attempts and do not want to use donor sperm. Obtaining a greater proportion of good-quality embryos improves the long-term reproductive prognosis of the couple, increasing the cumulative probability of live birth per each good-quality embryo transferred and reducing the need for additional ICSI cycles. Although it is a little retrospective study of 27 couples, 375 embryos were evaluated (of which 214 were subjected to genetic analysis), which serves to establish some initial conclusions. Further studies are needed in this line.

Nevertheless, the use of surgically retrieved sperm has been associated with an increased risk of embryonic aneuploidy [33]. This could be related to the higher proportion of spermatozoa with numerical chromosomal anomalies in males with some type of infertility [26, 30]. Several papers to date compare the embryo ploidy from couples with azoospermia [31, 32] and from patients with severe male infertility [26–28]. In reference to couples with severe male factor infertility, the proportion of embryos with sex chromosome abnormalities was significantly increased compared to embryos from couples with normal semen parameters regardless of oocyte origin or insemination technique [29]. Additionally, the total aneuploidy rate was significantly higher as sperm concentration decreased in 2008 couples who underwent assisted reproduction treatment [28]. Similarly, Kahraman's group [27] studied retrospectively the results of 326 cycles of 279 couples with different degrees of male infertility. They found that the euploidy rate was lower in those couples using testicular sperm, while it was similar in those with severe male factor (males with sperm concentration

below 5 million/ml) and normozoospermic couples when the female partner was less than 35 years old. Besides, the rate of embryonic mosaicism increased as male infertility became more severe. Nevertheless, this finding, they still commended the use of PGT-A in cycles with a clear severe male factor to prevent embryonic aneuploidy regardless of the woman's age.

However, as far as we know, none of the published studies evaluating the use testicular sperm instead of ejaculated sperm in non-azoospermic males has assessed the embryos ploidy status. This is an important issue because of the previously stated evidence of the relationship between severe male infertility and embryonic aneuploidy. In our current study, a similar aneuploidy rate was found after PGT-A in embryos of EJ-ICSI and TT-ICSI cycles performed in these couples. According to this result, we can confirm that the testicular origin of the spermatozoa does not increase the aneuploidy rate of embryos when it is compared within the same couple. However, we believe that genetic screening for embryonic aneuploidy is recommended when applying this clinical strategy in order to improve the reproductive prognosis of these couples.

With respect to clinical outcomes, eight of 27 couples finally obtain a newborn after the change to testicular sperm. The improvement in embryo quality after the change in the origin of the sperm may have led to an increase in the chances of having a newborn. It is true that the data we show refer to 60% of the couples included, since the follow-up of the remaining couples was lost. Even so, biochemical pregnancy was achieved in eleven women, of whom eight became ongoing pregnancy (>12 weeks of gestation). All resulted in the delivery of a live birth.

Improved reproductive outcomes in 17 couples with a clear severe male factor and unsuccessful ejaculated ICSI cycles were also previously reported with the Ben-Ami study [6], which found significantly higher implantation (20.7% vs 5.7%) and pregnancy rate (42.5% vs 15.1%), resulting in a greater proportion of couples with a child at home after switching to testicular sperm (27.5% vs 9.4%). Also importantly, the multivariate logistic analysis performed showed three major predictors of pregnancy, which in order of greatest to least impact were testicular sperm use, use of motile sperm, and the woman's age. However, we are unable to compare reproductive outcomes between the two types of interventions due to the lack of pregnancy outcomes in ICSI cycles with ejaculated sperm. Our results, in this case, are merely descriptive, as was done in the small Weissman's study [9], in which the switch to testicular sperm was an effective strategy in all four couples evaluated.

On the other hand, with this study design, we were allowed to control typical confounding factors, as patients served as their control (a paired statistical model was applied). As female age is one of the most important factors when evaluating embryo aneuploidy, in this case, no significant differences were found between the EJ-ICSI and TT-ICSI groups, with only 0.8 years difference between the first and the second ICSI attempt. The choice of analyzing two ICSI cycles closest in time helps to minimize the aging of the patient's oocytes. However, we cannot forget that the age of the woman in those older couples may be masking this finding despite the performance of PGT-A in both ICSI cycles. On the other hand, about 64% of the couples had a female infertility factor. This condition could be contributing in an unknown way the results presented here, not being the sperm factor the only one acting.

In parallel, some reports did not found an increased risk of adverse effects on neonatal and perinatal outcomes of children born by the use of testicular spermatozoa [5, 65, 66]. Nevertheless, others authors have suggested that the use of testicular sperm in couples with severe male factor may increase the genetic risk of the offspring [67] and recommended undergoing a PGT-A in this ICSI cycles [68].

Although the potential benefits of this clinical approach, it must be recognized that performing a testicular sperm retrieval is an invasive technique. In this study, all males had TESE to obtain the sperm cells. This procedure can be associated with complications during and after the extraction [64], which may condition the decision of patients to perform an ICSI cycle with testicular sperm having spermatozoa in their ejaculate. Therefore, couples should be properly counseled before proposing this strategy, assessing all the possible benefits in order to improve reproductive success and achieve patient's purpose, a take-home baby.

Limitations of the current study include its retrospective nature, which is always subject to bias of clinical practice, and the reduced number of patients included due to the selection criteria and how quite unusual this option is in ART centers because patients prefer to switch to donor sperm and avoid testicular sperm extraction. More well-designed and prospective controlled trials are needed to extrapolate the results to the population. Moreover, the technique used to assess chromosomal aneuploidy and the day of biopsy varied over time, which may have influenced the accuracy of the results obtained, although the technique used was the same in both ICSI cycles.

On the other hand, we aimed to evaluate the effect of sperm origin on embryos from ICSI cycles because it had not been adequately assessed to date. A strength of this study is that the comparisons between the EJ-ICSI and TT-ICSI groups were performed in the same couple, which allows controlling for potential confounders between both groups, adding value to the results obtained.

In conclusion, our data indicate that switching to testicular sperm improves the quality of available embryos without affecting their chromosomal load in couples with previous ICSI failure. Obtaining good quality embryos per stimulation

cycle initiated adds more chances of pregnancy, increasing the cumulative probability of live birth after their consecutive transfer and reducing the number of cycles required. Larger prospective and controlled studies are needed to confirm this finding, though. This clinical strategy may benefit the reproductive prognosis of non-azoospermic males but with poor semen parameters and with a previous ICSI failure who want to increase the chances of success in seeking parenthood and are reluctant to use donated sperm.

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Author contribution All authors contributed to the study conception and design. Irene Hervas, Maria Gil Julia, and Ana Navarro-Gomezlechon analyzed the data. Irene Hervas and Maria Gil Julia drafted the paper and prepared the manuscript. Nicolas Garrido revised critically. All authors provided the final approval of the completed manuscript.

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Declarations

Ethics approval This retrospective study was approved by The Ethics Committee for Investigation of the University and Polytechnic La Fe Hospital (project code 2011-FIVI-096-NG).

Consent to participate This study was performed with data obtained for clinical purposes. Informed consent was not obtained from the eligible subjects, as it was a retrospective study performed in the center where the data was obtained.

Consent for publication The authors are responsible for the correctness of the statements provided in the manuscript.

Conflict of interest The authors declare no competing interests.

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